INVENTION SUMMARY WITH PRINCIPLE DESIGN, DESCRIPTION AND REVINDICATION

TITLE

Therapeutic compositions for the treatment of skin diseases such as vitiligo, acne, psoriasis, alopecia and hypochromia.

SUMMARY

Descriptions of pharmaceutical compositions to be applied topically for the treatment of skin diseases such as vitiligo, acne, psoriasis, alopecia, and hypochromia. They are composed of one or more oils of animal origin, chosen from cod liver oil, mink oil and tortoise oil. Dissolved or dispersed in these oils are lithium, zinc, copper, and eventually gold, silver, sulfur and selenium silicon.

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Description of the aforementioned industrial invention:

Therapeutic compositions for the treatment of vitiligo.

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This invention deals with therapeutic compositions to be administered topically for the treatment of vitiligo.

Vitiligo is an alteration of the system of pigmentation, caused by the disappearance of melanocytes in characteristic skin areas.

The cause of such a disease still remains unclear, although intense research in this area has been completed in the past ten years.

In fact, the exact manner in which the melanocytes disappear, resulting in the loss of skin color, is an enigma. It is, in fact, hypothesized that chemical immunological factors, genetic or neural, may cause the onset of such a disease. Moreover, it is still not clear if vitiligo is a specific disease with specific causes, or if the loss of melanocytes is the consequence of the onset of more diseases. In any case, vitiligo strikes both sexes with an incidence rate of between 1% and 5% of the general population and at the moment there are no known therapeutic treatments able to diminish the depigmented skin zones. Nor are there any existing therapies able to overcome this type of disease.

On the other hand, it is known that many enzymes function only in the presence of small quantities of metal ions, contained in the enzyme molecules, and because of this they become defined as oligometals.

The most well-known of the various oligoelements that are present in enzymes, or that activate them, is certainly Iron, which is an integral part of Heme. Furthermore, it is present in numerous enzymes, such as cytochrome-oxidase, peroxidase and catalase. Another important oligoelement is copper, which conditions the proper use of iron on behalf of the organism. Moreover, it is also present in oxidized lysine and is involved in the metabolism of collagen and elastin. Zinc is another oligoelement found almost everywhere, as it is present in approximately 100 enzymes. Of these, some of the most important ones are dehydrogenase NAD, and NADP, polymerase DNA and RNA, carbonic anhydrase and peptidase carboxin.

Therefore, the oligoelements are very important from a chemical perspective, and also have an important therapeutic role.

Oligotherapy is the branch of medicine that deals with the treatment of various diseases by means of the oligoelements. Many skin diseases have been treated through oral administration of some oligoelements, receiving good results.

For example, psoriasis has been cured by administering Zinc, or salts of Li and Cu-Au-Ag, and also sulfur. The same salts of Li and Cu-Au-Ag, with sulfur treatments, are

used for the treatment of neurodermatitis, which are skin disturbances on a neurological level.

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Researchers have now found, unexpectedly, that it is possible to cure vitiligo in an efficient manner by the topical administration of the metals Cu, Li and Zn, and eventually Au and Ag. These are dissolved in or dispersed throughout an oil of animal origin, or in the corresponding ester alkaloid, chosen from mink oil, tortoise oil, cod liver oil or a mixture of oils.

In particular, researchers have verified that with the invention of these pharmaceutical compositions, even after one brief treatment, a swift repopulation of the morphologically and functionally normal melanocytes is observed in the areas affected by vitiligo.

Researchers have also found that the best results are obtained when not only lithium, copper and zinc are present, but also silver and gold.

The metals are generally present in the pharmaceutical compositions in the form of salts, complexes, or in the form of colloidal metal.

Preferably, the following salts are used: copper sulfate, silver lactate, gold and sodium sulfite, zinc, lithium and copper sulfate or gluconate.

Generally, the concentration of the single metals in the pharmaceutical compositions, according to this invention, is included in the following levels in the oil or mixture of oils:

Zn 7.10^{-4} - 0.387 mg/ ml of oil or oil mixture.

Li 10^{-4} - 6.2 mg/ ml of oil or oil mixture.

Cu 10^{-6} - 0.264 mg/ ml of oil or oil mixture.

Au $3. 10^{-8}$ - $3.5 .10^{-3}$ mg/ ml of oil or oil mixture.

Ag 3.10^{-8} - $5. 10^{-3}$ mg/ ml of oil or oil mixture.

It is preferable that the concentration of zinc falls between 10^{-3} and $7.7\ 10^{-2}$ mg/ml of oil or oil mixture, and the concentration of lithium falls between $1.5\ .10^{-3}$ and 10^{-2} mg/ml of oil or oil mixture. It is preferable that the concentration of copper falls between 10^{-5} and 5.10^{-4} mg/ml of oil or oil mixture, the concentration of gold falls between 2.10^{-6} and 4.10^{-5}, and the concentration of silver falls between 10^{-6} and 10^{-4} mg/ml. It is optimal that the metals in the form of salts, complexes or colloidals are added to the above-mentioned oils or oil mixtures in the form of a watery solution.

Therefore, it is preferable that the watery solution is present in concentrations between 3% and 1.8%, and even more preferable that it is 5% of the weight, in respect to the weight of the total pharmaceutical composition.

The animal oils used in the pharmaceutical compositions, and particularly mink oil which is habitually used in cosmetics, have the following characteristics in common:

They are natural, non-toxic, and are easily absorbed by the epidermis. They also spread easily on the skin and are stable.

In the pharmaceutical compositions, according to this invention, the metals are used as critically essential vectors in obtaining the desired therapeutic end result. Good results are obtained when the metals are dispersed in only one of the three cited oils or in a

mixture that contains only two. However, the best results are achieved when the metals are dispersed in a mixture containing all three oils. And from the previous pharmaceutical compositions listed, the one in which there is a volumetric ratio of 1:1:1 between the three oils is preferred.

In place of the oils, the relative ester alkali may be used, and preferably the _____.

In fact, these esters present an elevated ability and speed in penetrating the skin, in respect to the corresponding oils.

The pharmaceutical compositions, according to this invention, may be in the form of ointments or creams. They may also eventually contain other oligoelements, such as sulfur, silicon and selenium, and eventually other components such as Vaseline oil, refined coconut oil, excipients, tensioactives, etc.

Moreover, researchers have found that repigmentation is accelerated considerably if the application of the pharmaceutical composition is combined with a treatment of phototherapy.

Some illustrative, but not limitative, examples of the composition of this invention are listed below.

Preparation of composition 1.

1 ml of a phial from 2 ml BIOLGO® of SPECCHIASOL®, and a watery solution of the following salts:

Copper sulfate:	0.529	mg
Copper:	0.21	mg

Silver lactate	0.01	mg
Silver:	0.006	mg
Gold and Sodium sulfite:	0.003	mg
Gold:	0.0014	mg

2 ml of a watery solution of Zinc gluconate have been added, in which the concentration of the Zinc ion is 0.175 mg/ml. 2 ml of a watery solution of Lithium gluconate have also been added, in which the concentration of the Zinc ion is 0.25 mg/ml.

The watery solution (5 ml) obtained in this manner is dispersed in 95 ml of an equivolumetric mixture of mink oil, tortoise oil and cod liver oil.

The physical chemical characteristics of the oils used are reported in the following table:

See table 1(p.23)

The following is the concentration of oligoelements in the pharmaceutical preparation:

Zn $3.5 \cdot 10^{-3}$ mg/ml

Li $5 \cdot 10^{-3}$ mg/ml

Cu 1.05 $.10^{-5}$ mg/ml

Au 7 $.10^{-6}$ mg/ml

Ag 5 $.10^{-5}$ mg/ml

Composition 2

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Composition 2 was prepared in the analogous way described in composition 1, containing metals in the following concentrations:

Zn 1.4 . 10^{-3}

Li 2 . 10^{-3}

Cu 3 . 10^{-4}

Au 2 . 10^{-5}

Ag 2 . 10^{-6}

Description of the clinical experiment.

25 patients of both sexes underwent topical treatment, by means of weekly applications of composition 2, for a period of three months. The subjects were between the ages of 16 and 52 years old.

The extent of the disease varied noticeably, as did the amount of time that had passed since the onset of the disease.

In order to completely evaluate the efficacy of this treatment, the subjects in the study include both those who are recently ill, who have had the disease for several months, and those who are in the advanced stages of the disease, who have had it for years and are not responsive to conventional treatments.

In fact, recent cases cannot be considered pre-treated in the true sense, as past attempts have been sporadic and fruitless. The advanced cases underwent different cycles of therapy that alternated between treatments with a retinoic acid base and sessions of psoralen and phototherapy.

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The results of the subjects' attempts were so disappointing that both the patients and their doctors wanted to discontinue the treatment. The extent of the disease varied from one or two vitiligo areas situated in the mental region, to massive segmentary interest of the superior and inferior veins, as well as the trunk on the median line, the neck and the region of the genitals. Having to quantify area of interest, the observed areas on the treated cases varied from 4 cm^2 to more than 90% of the bodily surface.

The base treatment plan oversaw the topical application of composition 2, which was uniformly distributed on the depigmented area, followed by a session of phototherapy focused on the same area.

This plan was repeated for a maximum of three weekly sessions for four weeks. To successfully accelerate the therapeutic effect, the criteria of applying the product daily to the de-pigmented zones was adopted. Furthermore, subjects were encouraged to expose themselves to sunlight, also continuing with the usual sessions of phototherapy. In order to find the optimum therapeutic composition, some patients—not including the 25 described here—were treated for four weeks with a preparation missing one or more of the fundamental elements already listed.

After the test period, the treatment was stopped and subsequently altered, taking a necessary interval to evaluate the eventual therapeutic results, according to the way described and using the complete preparation.

In particular, through conducting these comparative clinical tests, it has been observed that:

a) the absence of one or two of the animal oils (tortoise, mink or cod liver oil) noticeably slowed the process of re-pigmentation.

b) the absence of one or two of the oligoelements, such as copper, zinc and lithium, reduced or abolished the total therapeutic effect.

c) the preparation containing the three oils and the oligoelements Zn, Cu and Li, but without the oligoelements silver and gold, had a noticeable therapeutic effect. Nevertheless, it was inferior to the preparation that included them.

d) the phototherapy and/or the exposure to sunlight noticeably accelerated the re-pigmentation process, while the use of the preparation alone, or its application to areas constantly protected from sunlight, slowed the reaction time.

The re-pigmentation of areas treated only with composition 2, that is, without exposure to phototherapy, occurred more slowly, but presented the same clinical and histological characteristics as the areas that underwent the combined treatment.

The results obtained with the final preparation may, therefore, be summarized in the following manner:

Taking into account the obvious individual biological differences between patients and the diverse stages of the disease, it is observed that three months after beginning treatment, the de-pigmented areas with minor diameters of between 2 and 4 cm were completely re-pigmented. In this case, the re-pigmentation clinically appears to advance from the borders of the lesions to the confluence of re-pigmentation nuclei in the center of the lesion.

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On the other hand, in vast areas of vitiligo, for example, the complete de-pigmentation from the distal phalanxes of the fingers to the bend of the elbow, the re-pigmentation followed an analogous pattern, advancing from the front of the normally pigmented skin. However, it appeared particularly significant that the insurgence of multiple passages of re-pigmentation, clinically perifollicle, with an evolution towards the confluence, were produced in areas where the migration of melanocytes from surrounding normally pigmented areas wouldn't ordinarily be possible.

In percentage terms, the response to the treatment was 100%, as all of the patients showed a positive therapeutic effect ascertained by the reappearance of melanocytes and pigment in areas long affected by vitiligo. Moreover, it has been revealed that the process of re-pigmentation follows the stages that characterized the progress of the disease in reverse, in each and every patient. In fact, the first areas to regain pigment are those recently formed by the disease, while the older areas responded to the process of re-pigmentation more slowly. The extent of the individual response is naturally conditioned by three factors:

a) the regularity of the application of the preparation.

b) the frequency of the phototherapy treatments or exposure to sunlight in combination with the application of the preparation.

c) the duration of the therapy.

On the other hand, only the exposition to sunlight or phototherapy treatments induced an intense inflammation, not followed by re-pigmentation.

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The intense re-pigmentation observed in patients that underwent treatment with composition 2 showed that this composition was able to induce, in early periods of therapy, the presence of melanocytes functioning in previously de-pigmented zones. By the third month of therapy, it was able to restore pigment in a high percentage of individual patients.

Morphological research from samples collected from 5 patients affected by vitiligo and in the process of treatment with composition 2 and radiant therapy:

Morphological research was conducted from skin samples from 5 patients only three months into the treatment of composition 2 and radiant therapy.

In particular, biopsies were performed on areas 6 mm in diameter, upon appropriate anesthetization and gentle perpendicular stretching in the direction of the lines of tension in the previously chosen skin zone.

As general criteria, the skin zone within the ring of inflammation was chosen, present in each patient within the vitiligo mark and the neonormopigmented margin.

The characteristics are reported on the following table:

(All patients, regardless of age or sex, underwent a biopsy)

TABLE

CASE #	AGE	SEX
1	24	M
2	18	F
3	14	F

4	33	F
5	43	M

Each bioptic piece was immediately divided into two samples, reserving the biggest portion for histological research, which was then immediately immersed in a solution of 40% formaldehyde at a neutral pH of 10%. The remaining part of the sample was divided further into smaller pieces of approximately 1 mm^3 and immediately immersed in Karnowsky liquid in order to obtain an adequate reading for ultrastructural study.

The portion of the sample reserved for research under an optical microscope is then halved. One half is enclosed in metacrilate and the other half in paraffin.

The sections in paraffin, with a thickness of 6/7 microns, were colored either by eosin hematoxilin (Em-Eos), or by Masson Fontana's method (M-F), which uses a solution of ammoniacal silver nitrate able to demonstrate melanin's silver-affinity. This is able to reduce silver's salts by the liberation of metallic silver (black-brown in color) that is deposited at the level of a substance reducer.

The sections enclosed in metacrilate, with a thickness of 2 microns, were colored either by Em-Eos, or by means of Gomori's technique, which is a solution of silver hexamethylene-diamine (Ag-Mot). This technique shows the silver-affinity of granules of melanin, eventually present in the form of melanocytes and keratinocytes present in the sample sections.

The small pieces were processed according to the normal electronic transmission microscope routine.

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In particular, the specimens that were subjected to the electronic microscope, after being fixed in Karnovsky liquid and washed on a phosphate pad, were then fixed in osmium tetroxide and dehydrated to a mass reduced to acetone, before being included in epoxidic Araldite® resin. After polymerization at a heat of 56°C for at least a week, the samples were prepared on a glass plate with semi-fine sections (with a thickness of 1 micron), and colored with blue methylene and saffron in order to choose the best mode for ultra-structural study. For each case, 5 samples were prepared this way.

Finally, after the last samples were cut with the ultramicrotom, using a diamond blade for the ultra-fine sections, they were colored with heavy metal salts before being observed under the electronic microscope.

OPTICAL MICROSCOPE

The cytomorphological characteristics of the melanocytes are evident only by means of special colorization; In fact, in the sections that were obtained by either inclusion in paraffin or inclusion in metacrilate and colored by Em-Eos, the melanocytes may be

recognized because they are presented in the form of small, round, cellular elements with a small, strongly-colored nucleus. Also, they display clear cytoplasm followed by coartation. Because of this, melanocytes are defined as "clear cells of the basal layer." However, it is good to specify that not all of the clear cells (whether of the basal layer or, above all, of the other layers) are melanocytes , since other cells can be coartated in the course of preparing the tests. It is also good to specify that the best histochemical

way to emphasize the melanocytes is the After-reaction to Block applied on a sheet of epidermis, removed from the dermis through tripsinization.

In this manner, it is possible to emphasize the cells that possess the enzyme tyrosine—which are only melanocytes—on non-fixed tissue.

It is also good to emphasize that it would have been opportune to practice on seriate sections—like those on which the colorizaton was done to demonstrate melanin's silver-affinity—a decolorization with silver oxidants (for example, water oxygenated to 10%) in order to obtain a further confirmation of the presence of pigment. In fact, the other pigments eventually present would remain unaltered from the action of the oxidant agent, which would be a further confirmation that the characteristic black-brown color of the granules is undoubtedly due to the presence of melanin.

Case 1 – (male – 24 years old – back of the left hand)

The following test-plates were prepared:

1) Paraffin—colored with: Em-Eos(2) – see photo # 1

" " " M-F (4) – see photo # 2

2) Metacrilate—colored with: Em-Eos(2) –

" " " Ag-Met(4) – see photos # 3,4,5,6

3) Semi-fine Araldite - see photo # 7

The two examined skin fragments were distinguished, the epidermis from the dermis, by orthological parameters.

The "clear cells of the basal layer" are easily found on the test-plate including paraffin and colored with Em-Eos. See photo # 1 (x460).

Photo # 2 shows the silver-affinity of the melanin pigments; which are better seen in the preparations including metacrilate and seen in photos 3 (x460), 4 (x730), 5 (x460) and 6 (x730). Furthermore, they demonstrate the methodical use of a silver hexamethylene-diamine solution (Ag-Met).

Photo # 7 shows the area pre-chosen for the ultra-structural study; colored with methylene blue and saffron (x460).

Case 2 (female – 18 years old – back of the left hand (?))

The following test-plates were prepared:

1) Paraffin—colored with: Em-Eos(2)

" " " M-F (4) – see photos # 8 and 9

2) Metacrilate—colored with: Em-Eos(2) – see photo # 11

" " " Ag-Met(4) – see photos # 10 and 12

3) Semi-fine Araldite - see photo # 13

Normal skin in its parameters. In the specimens, it is easy to distinguish "clear cells of the basal layer," interpreted most often as melanocyte cells. The presence of grains of melanin is confirmed by the methodical histochemistry of Masson Fontana, which is ammoniacal silver weakly contra-colored. See photo # 8 (460).

Because of this, figures 10 and 12 of the tests including metacrilate show evidence of the silver-affinity of melanin, with the methodical use of a solution of silver hexamethylene-diamine (Ag-Met) and, respectively, x460 and x1840.

Photo # 13 shows the area pre-chosen for the ultra-structural study under the electronic microscope.

Case 3 (female – 14 years old – back of the left hand)

The following test-plates were prepared:

1) Paraffin—colored with: Em-Eos(2)

" " " M-F (4)

2) Metacrilate—colored with: Em-Eos(2) – see photo # 16

" " " Ag-Met(4) – see photos # 14 and 15

3) Semi-fine Araldite - see photo # 17

In the context of a square of normal skin, the tests treated with the common colors, seen in figure 16 (x460), don't draw attention to anything. On the other hand, in the sections

treated with methodic histochemicals to show the silver-affinity of the granules of melanin, a continuous black outline that tinges all of the cells of the basal layer is clearly present, as seen in photographs 14 (x460) and 15 (x1840).

Photo 17 shows the area of study chosen for the electronic microscope.

Case 4 (female – 33 years old – back of the left hand)

The following test-plates were prepared:

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1) Paraffin—colored with: Em-Eos(2) – see photo # 20

" " " M-F (4) – see photos # 18 and 19

2) Metacrilate—colored with: Em-Eos(2) –

" " " Ag-Met(4)

3) Semi-fine Araldite - see photo # 21

In the context of skin within normal parameters, numerous "clear cells of the basal layer" appear in paraffin in the preparation, colored with Em-Eos (see photo # 20, x730) in the Masson Fontana method. In these last ones it is very evident that the silver-affinity of the granules of melanin is present in large numbers in the deep layers of the epidermis. (See photos 18 x730 and 19 x730).

Photo # 21 shows the area of study chosen for the electronic microscope.

Case 5 (male – 43 years old- (back of the left hand))

The following test-plates were prepared:

1) Paraffin—colored with: Em-Eos(2) – see photo # 24

" " " M-F (4) – see photos # 22 and 26

2) Metacrilate—colored with: Em-Eos(2) – see photo # 27

" " " Ag-Met(4) – see photo # 28

3) Semi-fine Araldite - see photo # 32

In all of the test-plates, skin within normal parameters is observed. The clear cells of the basal layer are particularly evident in the samples including paraffin, either with Em-Eos (see photo # 24) or with ammoniacal Ag (the Masson Fontana method), as it may be seen

in photo # 26. In photo # 22, a very slight contra-coloration was performed in order to emphasize the brown-black color of the melanocyte pigments able to reduce silver's salts. This silver-affinity is documented here also in photos # 27 and 28, and in preparations including metacrilate.

Photo # 32 shows the area of study chosen for the electronic microscope.

The morphological study described above, conducted on samples of vitiligo skin prevalent in patients only three months into treatment, emphasized the following outline:

- The presence of "clear cells at the basal level" in preparations including paraffin and colored with Em-Eos.
- The intense silver-affinity in melanin pigments, verified either in preparations including metacrilate and treated with silver hexamethylene-diamine, or in melanocytes or keratinocytes.
- Presence of melanin granules in preparations including paraffin and treated with the histochemical method of Masson Fontana, in ammoniacal silver.

- Normal skin parameters: in particular, the absence of acanthosis phenomenon and hyperkeratosis, normally found in the skin of patients treated with traditional methods.

REVINDICATION

1. Pharmaceutical compositions to be used topically for the treatment of vitiligo, composed of an oil of animal origin or from its corresponding ester alkaloid, chosen from

cod liver oil, mink oil, tortoise oil or a mixture of them, in which the following metals are dispersed or dissolved:

Lithium, copper, zinc, and eventually gold and silver.

2. The pharmaceutical compositions according to Revindication 1, characterized by the fact that they also contain the metals gold and silver.
3. The pharmaceutical compositions according to Revindication 1 and 2, characterized by the fact that the metals are present in the pharmaceutical composition, in the form of salts, complexes or colloidal metals.
4. The pharmaceutical compositions according to Revindication 3, characterized by the fact that the metals are present in the form of the following salts: copper sulfate, silver lactate, gold and sodium sulfite, zinc, lithium and copper sulfate or gluconate.
5. The pharmaceutical compositions for the treatment of vitiligo according to Revindication 1, where the concentration of metals in the oil, or the mixture of oils, is at the following intervals:

Zn 7.10^{-4} - 0.387 mg/ml of oil or oil mixture

Li 10^{-4} - 6.2 mg/ml of oil or oil mixture

Cu 10^{-6} - 0.264 mg/ml of oil or oil mixture

Au 3.10^{-8} - $3.5 .10^{-3}$ mg/ml of oil or oil mixture

Ag 3.10^{-8} -$5. 10^{-3}$ mg/ml of oil or oil mixture

6. The pharmaceutical compositions according to Revindication 5, characterized by the fact that the concentration of zinc is between 10^{-3} and $7.7\ 10^{-2}$ mg/ml of oil or oil

mixture, the concentration of lithium is between $1.5 .10^{-3}$ and 10^{-2} mg/ml of oil or oil mixture, the concentration of copper is between 10^{-5} and $5. 10^{-4}$ mg/ml of oil or oil mixture, the concentration of gold is between 2.10^{-6} and 4.10^{-5} mg/ml and the concentration of silver is between 10^{-6} and 10^{-4} mg/ml.

7. The pharmaceutical compositions for the treatment of vitiligo according to Revindication 1, characterized by the fact that the metals are dispersed or dissolved in a mixture of three oils: cod liver oil, tortoise oil and mink oil.

8. The pharmaceutical compositions according to Revindication 7, in which the three oils are present in a volumetric ratio of 1:1:1.

9. The pharmaceutical compositions for the treatment of vitiligo according to Revindication 1, characterized by the fact that the metals are dispersed in the ester alkali of mink, tortoise and cod liver oils.